Acceptability and Suitability of Athel, *Tamarix aphylla*, to the Leaf Beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a Biological Control Agent of Saltcedar (*Tamarix* spp.)

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Environ. Entomol. 35(5): 1379–1389 (2006)

ABSTRACT The leaf beetle *Diorhabda elongata* (Brullé) *sensu lato* has been released in the western United States for the classical biological control of exotic saltcedars (*Tamarix* species and hybrids). However, athel (*T. aphylla* [L.] Karsten), an exotic, moderately valued evergreen species in the southwestern United States and northern Mexico, has not been targeted for biological control. All populations of *D. elongata* previously examined, including those promising for release in southern areas of the saltcedar infestation, develop and oviposit on athel. Therefore, we assessed more fully the acceptability and suitability of athel to three *D. elongata* populations (Tunisia, Crete, and Uzbekistan). All populations of *D. elongata* laid similar numbers of eggs on athel and saltcedar in no-choice tests. In multiple- and paired-choice tests, oviposition on saltcedar was generally greater than on athel but with some notable exceptions and inconsistencies within populations. Increasing cage size delayed the colonization of and oviposition on test plants by small groups of adult beetles but did not change the pattern of egg-laying. For Crete beetles, survival and development were similar for larvae fed athel or saltcedar. Adult size was negatively affected by a larval diet of athel. An adult diet of athel did not reduce lifetime fecundity, although it did decrease egg mass size and delayed the start of oviposition. As a result, the innate capacity for increase decreased. The potential for damage to athel by *D. elongata* may be higher than previously thought; however, this may be offset by the potential for increased invasiveness of athel.

KEY WORDS weed biological control, *Diorhabda elongata*, athel, saltcedar, *Tamarix aphylla*

Ten species of *Tamarix* L. (Tamaricales: Tamaricaceae) were introduced into North America beginning as early as 1823 for use as ornamentals, for windbreaks and shade, and to stabilize stream banks (Horton 1964, Baum 1967, Crins 1989, DiTomaso 1998). After the late 1920s, some of these species became invasive along river ways, reservoirs, lakeshores, and desert springs in the western United States and northern Mexico (Robinson 1965, DeLoach et al. 2000). The invasive entity is currently comprised of a complex of four or five species and various hybrids of saltcedars, which are cold-, drought-, fire-, and salinity-tolerant deciduous shrubs or small trees (Gaskin and Schaal 2002, 2003). A biological control program was initiated against saltcedar by the USDA–ARS at Temple, TX, in 1986. After extensive host-specificity testing, the leaf beetle *Diorhabda elongata* (Brullé) *sensu lato* (Coleoptera: Chrysomelidae) was released, with populations from China and Kazakhstan established in northern areas of the Unites States and populations from Greece having been released in 2003 and 2004 at select locations south of latitude 37° N (DeLoach et al. 2003, 2004, Lewis et al. 2003a, 2003b). Additional populations also have been tested that would likely establish at southern latitudes in North America (Milbrath and DeLoach 2006, J. C. Herr, unpublished data). However, no releases for saltcedar biological control currently have been made near the U.S.–Mexico border because of potential conflicts involving *Tamarix aphylla* (L.) Karsten, which is commonly called athel.

Athel is one of the introduced *Tamarix* species that has not been targeted for biological control and is generally not considered invasive in North America at present (DeLoach et al. 2003), although it is highly invasive in Australia (Griffin et al. 1989). Athel is a tree-sized, evergreen species that is cold-intolerant, being found from northern and eastern Africa to Pakistan (Baum 1978). In North America, it has been planted in Arizona, California, Nevada, New Mexico, Texas, and Utah, as well as the northern Mexican states of Baja California, Chihuahua, Coahuila, Durango, Nuevo Leon, Sinaloa, Sonora, and Tamaulipas (Baum

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1967, González and Aldape 1991, DeLoach 2004, USDA–NRCS 2005). Athel is still considered to be of some value in parts of the southwestern United States as an ornamental shade tree or as a windbreak, although it has generally not been recommended in recent years because of its nature of dropping large quantities of brittle limbs and twigs (DeLoach et al. 2003). Athel is of greater value in desert areas of northern Mexico, being both a drought-tolerant and large shade tree, reaching 20 m in height (DeLoach et al. 2003). For these reasons, athel was designated a critical test plant for the saltcedar biological control program, i.e., a species within the order Tamaricales on which only a low level of damage is acceptable (DeLoach et al. 2003). Previous host-specificity testing of *D. elongata* indicated that athel was suitable for larval development, but the females that were produced laid much fewer eggs than females produced from larvae fed only saltcedar (Lewis et al. 2003b). Also, athel may be less preferred than saltcedar for oviposition, with females of most populations often laying one third the number of eggs on athel than saltcedar in choice tests (DeLoach et al. 2003, Lewis et al. 2003a, Milbrath and DeLoach 2006). However, it is unclear how relatively less oviposition in cage tests equates to damage in the field (Arnett and Louda 2002) or what degree of host acceptance of athel will occur in the field in the absence of saltcedar.

Most athel stands in the United States and Mexico are the result of deliberate plantings, with few naturalized stands known initially (DeLoach et al. 2003). More naturalized stands have been observed in recent years (C.J.D., unpublished data), presumably through vegetative spread from broken branches after floods (Danin 1981). However, successful reproduction by seed has recently been reported along the shores of Lake Mead, NV (Barnes 2003), which is also the primary means of spread in Australia (Griffin et al. 1989). In addition, hybridization between athel and the invasive saltcedars *T. ramosissima* Ledebour and *T. chinensis* Loureiro has been confirmed at three sites in the southwestern United States (Gaskin and Shafroth 2005). Thus, athel or an athel hybrid has the potential to become invasive, which would create a future conflict of interest in minimizing damage to athel, one of the original goals of the saltcedar biological control program.

We therefore evaluated the acceptability (or attractiveness) of athel for colonization and oviposition by adult *D. elongata* from Crete, Greece; Karshi, Uzbekistan; and Sfax, Tunisia, under no-choice and choice conditions in outdoor cages, including cages of different sizes. In addition, we examined the ability of the Crete population to complete its larval and adult development on athel (suitability), including athel's effect on beetle reproduction.

Materials and Methods

Insect Colonies

The three populations of *D. elongata* included in this study originated from North Africa to central Asia. They were collected 15 km south of Sfax, Tunisia (latitude 34.66° N, longitude 10.67° E, elevation 10 m); 3 km west of Sfakaki, Crete, Greece (latitude 35.83° N, longitude 24.6° E, elevation 7 m); and 7 km west of Karshi (Qarshi), Uzbekistan (latitude 38.86° N, longitude 65.72° E, elevation 350 m). The *Diorhabda* beetles collected on *Tamarix* in Asia and the Mediterranean area were all identified as *D. elongata* by A. S. Konstantinov (USDA–ARS, Systematic Entomology Laboratory, Beltsville, MD) and/or I. K. Lopatin (Byelorussian University, Minsk, Belarus). Ongoing research by our team indicates the probability of four closely related species in the biological control program. We here refer to these populations as *D. elongata* from Tunisia, Crete, and Uzbekistan. A fourth population collected near Turpan, China, that previously had been studied by us (Milbrath and DeLoach 2006) was not included because poor overwintering in central Texas seemed to preclude its usefulness in far southern sites where athel would occur (unpublished data). Voucher specimens of *D. elongata* were deposited with the National Collection of Insects and Mites of the National Museum of Natural History, Smithsonian Institution, Washington, DC (under lot no. GSWRL-2004-02).

Beetles were imported into the quarantine facility of the USDA–ARS Exotic and Invasive Weed Research Unit at Albany, CA, where parasites, predators, and pathogens were eliminated. Eggs and/or adult beetles were sent to the USDA–ARS Arthropod Containment Facility (quarantine) at Temple, TX, to initiate colonies or for immediate use in some tests. Beetle colonies were maintained in outdoor field cages on various planted saltcedars under a permit from USDA Animal and Plant Health Inspection Service received in 1998 and renewed in 2000.

Test Plants

Plants for our tests included various accessions from Arizona, Colorado, New Mexico, and Texas of athel and the saltcedars *T. canariensis* Willdenow/*T. gallica* L. (these two species are difficult to separate; Crins 1989, Gaskin and Schaal 2003), *T. parviflora* de Candolle, and the hybrids *T. ramosissima* x *T. chinensis* and *T. ramosissima* x *T. canariensis*/*T. gallica.* These plants were selected based in part on the availability of sufficient plant material. The species *T. ramosissima* and *T. chinensis* had been included in previous tests of the three beetle populations (Milbrath and DeLoach 2006). All *Tamarix* were identified by J. Gaskin (USDA–ARS, Sidney, MT) using the fourth intron of the nuclear phosphoenolpyruvate carboxylase (pepC) gene (Gaskin and Schaal 2002).

Plants were propagated from cuttings obtained from cultivated or naturalized *Tamarix* plants. Cuttings were planted in 8-liter pots containing a mixture of 10:3:2:1 parts of vermiculite, potting soil, peat moss, and sand and were fertilized twice yearly with pellets of a slow-release fertilizer (15–9-12 N-P-K). Plants were held under natural daylengths and 24–35°C in a

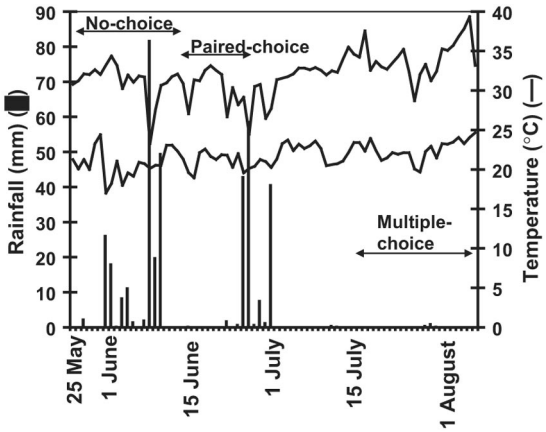

Fig. 1. Rainfall and minimum and maximum air temperatures during tests of *D. elongata* in outdoor cages at Temple, TX, 2004.

greenhouse or natural temperatures in an outdoor slathouse before their use in tests.

Adult Tests

Adult *D. elongata* host plant selection and oviposition preferences (acceptability) among athel and different saltcedar species and hybrids were studied using no-, paired-, and multiple-choice tests. In addition, the effect of cage size was assessed for the no- and paired-choice tests. All tests were conducted outdoors on the fenced grounds of the Temple ARS laboratory in 3 by 3 by 2-m (length by width by height) field cages under natural temperatures and daylengths (Fig. 1). The area immediately surrounding the field cages was treated periodically with hydramethylnon (Amdro; Ambrands, Atlanta, GA) to control fire ant (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) infestations. Plants inside the cages were watered with a drip irrigation system.

No-Choice Test. This test focused on adult colonization of and oviposition on athel and saltcedar when the test plants were offered individually. Tests were conducted outdoors from May to June 2004 (Fig. 1). The experimental arenas were 68 by 53 by 85-cm (length by width by height) aluminum screen cages placed inside the 3 by 3 by 2-m field cages. One potted saltcedar plant (*T. ramosissima* × *T. chinensis*, Pueblo, CO, accession) or one potted athel plant (Encino, TX, accession) was randomly assigned to a cage. The GenBank accession no. AY090385 and AY090386 represent the two haplotypes of the heterozygous pepC gene for the hybrid saltcedar used in this and the next test. Average heights (including a 20-cm-tall pot) of the plants were 70 cm. Pots rested on a 3-cm layer of potting soil that absorbed excess water within the cages to prevent the entrapment and drowning of beetles.

The experimental design for adult colonization included four factors in a repeated-measures design: three *D. elongata* populations (Crete, Tunisia, and Uzbekistan) and two test plants (athel or saltcedar) in a two-way treatment structure in a completely randomized design, with repeated measures on day postrelease (1, 2, and 3) and three locations for adults (test plant, cage walls, and dead/unaccounted). For oviposition, the experimental design was similar but only involved three factors: *D. elongata* population, test plant, and location of eggs (plant or cage walls). Five replications were used. Beetles (10 males and 10 females per cage) were released into the center of the cage. Adults were counted 1, 2, or 3 d after release, and their location was recorded. Adults not easily observed for the 1- and 2-d postrelease counts were scored as unaccounted to avoid unnecessarily disturbing the beetles. Because the 2-d counts were generally similar to the 1-d counts, they will not be presented. Adults were aspirated off the plants, and eggs were removed, noting their location, after 3 d. All plants were thoroughly washed with water, and their locations were randomized between replicates. Two different sets of plants were used in alternate replicates. New adults were used for each replicate.

Paired-Choice Test. This test, conducted in June 2004 (Fig. 1), studied the preference by adult *D. elongata* between saltcedar and athel when offered together. One potted saltcedar plant (*T. ramosissima* × *T. chinensis*, Pueblo, CO) and one potted athel plant (Encino, TX) were paired in each cage. The location of each pot within a cage was randomly assigned. Average heights (including the 20-cm-tall pot) of the plants were 70 cm. The experimental design for adult colonization involved a one-way treatment structure with repeated measures on day postrelease (1, 2, and 3). Each treatment was a combination of a beetle population (Crete, Tunisia, or Uzbekistan) and a location of adults (saltcedar, athel, cage walls, or dead/unaccounted). The experimental design for oviposition involved a one-way treatment structure, with each treatment being a combination of beetle population and a location of eggs (saltcedar, athel, or cage walls). Five replications were used per treatment. Groups of 10 male and 10 female beetles were released into each cage. Adults and eggs were counted as previously described.

Cage Size. Simultaneously during the conduct of the previous two tests in small (0.3 m^3) cages, groups of 20 (10 male and 10 female) adult *D. elongata* from Crete were released into large (3 by 3 by 2 m, 18 m^3) cages, using similar test plants, placement of plants, and methods, to assess the effect of cage size on the response of *D. elongata* to athel and/or saltcedar in a no-choice and paired-choice setting. The experimental designs were similar to the previous tests except that cage size (small and large) replaced beetle population. Five replications were used.

Multiple-Choice Test. A multiple-choice test of adult *D. elongata* host preference among two athel accessions and four saltcedar species and hybrids was conducted from July to August 2004 (Fig. 1; see Table 5 for names). The experimental design was a one-way treatment structure with five or six replications per treatment. Each treatment was a combination of a *D.*

elongata population (Crete, Tunisia, or Uzbekistan) and a location of adults or eggs (six different test plants, cage walls, or ground).

Tests were done in the large field cages. Each cage was subdivided into four quadrats, each quadrat containing one each of the test plants in pots. The plants were randomly arranged in each quadrat of each cage, and the pots were placed on the soil surface. The average height of the plants (including the 20-cm-tall pot) ranged from 119 to 176 cm. For each beetle population, 62–129 unsexed adults were released in the center of a cage. After 3–6 d, the number of adults and eggs on each test plant and the cage walls and ground were recorded for each quadrat. Eggs were removed, and adults were aspirated off the plants. All plants were thoroughly washed with water and rotated one position clockwise within quadrats and between replicates to control for positional effects. Adults, previously used and new, for each population were released back into the same cage. The data analyzed were the average of the four quadrats for each observation date. Because of the variable number of adults available between beetle populations and replicates and the unknown proportion of females, adult colonization and oviposition data were converted to the percent of adults or eggs, respectively, found at each location for a given replicate.

Larval Development and Adult Fecundity

A no-choice test was conducted to compare the suitability of a saltcedar or athel diet on the survival, development, size, and reproduction of *D. elongata* from Crete. This population was chosen for testing as it already had been released in the field at various research sites beginning in 2003. The test was conducted from June to October 2004 in the quarantine laboratory at 28°C (range, 22–33°C) and a photoperiod of 16:8 h (L:D). Saltcedar foliage (*T. ramosissima* × *T. chinensis*, Artesia, NM, GenBank accession no. AY090385 and AY090386) was collected from plants growing on the laboratory grounds at Temple and stored in plastic bags with moist paper towels at 5°C. Harvested foliage was 0–7 d old when fed to the beetles. Athel branches were collected near Mercedes, TX, every other week and shipped overnight to Temple. Branches were washed with water and stored in 19-liter buckets, the cut ends under water, at 5°C. Larvae or adults were fed athel foliage that was 0–14 d old.

The first part of the test focused on the influence of diet on larval/pupal development and survival and adult size attained by *D. elongata.* Egg masses of Crete *D. elongata* were randomly divided among petri dishes, and cut foliage of either saltcedar or athel was placed in each dish. On hatching, 100 randomly selected larvae (0–15 h old) from the saltcedar dishes and 100 larvae from the athel dishes were placed in separate 50-ml ventilated, clear plastic vials. The experimental design therefore consisted of a one-way treatment structure (two types of diet) in a completely randomized design. Each larva was fed the same diet of excised leaves for its entire immature development. Leaves were replaced every 2–3 d, and larvae or pupae were checked daily for survival and development. Sand was placed in the bottom of each vial when mature third instars (final instar) were present to provide a pupation site. The vials were cleaned of all plant material before adult eclosion. Each larva served as a replicate (up to 100 per diet) for the duration of the various life stages. Percentage survival to the adult stage was calculated by randomly assigning 10 larvae to each of 10 groups (replicates) for each larval diet.

Adult beetles that eclosed were sexed using the characters of the last visible abdominal sternite (Lewis et al. 2003b). Adults were frozen immediately after eclosion if they were not used in the mating study or at death and measured for size. We measured the length of the left elytrum to the nearest 0.1 mm using an ocular micrometer at ×10. A highly significant linear relationship existed between elytral length and total body length (body = 1.325 × Elytrum, $R^2 = 0.99$, $F_{1,128} = 52{,}543.7$, $P < 0.0001$), allowing us to infer differences in body length. The analysis for this parameter included two factors—larval diet and sex of the adult beetle.

The second part of the test examined how different combinations of larval and adult diets affected the fecundity, oviposition schedule, and longevity of adult *D. elongata.* The experimental design involved a two-way treatment structure in a completely randomized design, with two levels of larval diet and two levels of adult diet (athel or saltcedar). The four treatment combinations were thus (1) larval and adult diet of saltcedar, (2) larval diet of saltcedar and adult diet of athel, (3) larval and adult diet of athel, and (4) larval diet of athel and adult diet of saltcedar. For each treatment, a mating pair of recently eclosed adults (<24 h old) from the previous experiment was placed in each of 15 clean vials. For each pair, males and females were of the same age and had originated from different egg masses. Foliage was replaced every 2–3 d. Adults were checked daily for survival and the presence of eggs. Males that died were replaced by similar-age males if available. Egg masses for each female were removed and placed in individual wells of marked well plates. The plates were covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL), in which pin holes were made to provide ventilation and held until hatching. The total number of eggs and hatched eggs per mass were recorded. The earliest egg masses also were checked twice daily to estimate the number of days until hatch. The preoviposition and oviposition period, total fecundity, and fertility were calculated for each female. The few females that escaped were not included in analyses. Total longevity was calculated for males and females. Based on the data obtained from the fecundity study and the larval/pupal development and survival study, we calculated population growth statistics, including net reproductive rate (R_0), mean generation time (T), innate capacity for increase (r_m), and population doubling time (DT), using methods described by Birch (1948). Com-

Table 1. Distribution of adult *D. elongata* (mean ± SD) from three different populations 1 and 3 d after release in no-choice tests with either saltcedar or athel: Temple, TX, May–June 2004

Location of adults	No. adults		
	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan
1 d after release			
Plant	15.1 ± 2.3ab	15.4 ± 2.1a	12.8 ± 3.2b
Cage walls	1.2 ± 1.2b	1.7 ± 1.5b	3.7 ± 2.5a
Dead/unaccounted	3.7 ± 2.7a	2.9 ± 2.0a	3.5 ± 1.5a
3 d after release			
Plant	17.1 ± 2.5a	15.8 ± 3.1a	14.5 ± 3.9a
Cage walls	2.7 ± 2.4a	3.1 ± 2.6a	4.1 ± 3.1a
Dead/unaccounted	0.2 ± 0.4a	1.1 ± 2.5a	1.4 ± 2.2a

Outdoor tests in small screen cages, each cage with 20 beetles (10 males, 10 females) and one test plant (saltcedar, *T. ramosissima* × *T. chinensis*, or athel, *T. aphylla*), $n = 5$. Means are averaged over saltcedar and athel, which was not a significant factor (test plant, $P > 0.05$). For each day postrelease and location, means within a row followed by the same letter are not significantly different (Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

Table 2. Distribution of adult *D. elongata* (mean ± SD) from three different populations 1 and 3 d after release in paired-choice tests with saltcedar and athel: Temple, TX, June 2004

Location of adults	No. adults		
	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan
1 d after release			
Saltcedar	10.2 ± 4.0a	9.0 ± 4.8ab	8.0 ± 1.2a
Athel	2.8 ± 1.8de	5.4 ± 4.7bcd	3.2 ± 2.4cde
Cage walls	2.6 ± 3.1de	1.0 ± 1.2e	2.6 ± 1.9de
Dead/unaccounted	4.4 ± 2.5cd	4.6 ± 1.5bcd	6.2 ± 0.8abc
3 d after release			
Saltcedar	14.0 ± 1.6a	10.2 ± 4.0ab	8.6 ± 2.7abc
Athel	4.0 ± 1.4def	5.6 ± 2.9cde	2.8 ± 2.4fgh
Cage walls	1.8 ± 1.1ghi	3.2 ± 2.7efg	6.2 ± 2.6bcd
Dead/unaccounted	0.2 ± 1.4i	1.0 ± 1.0hi	2.4 ± 2.1fgh

Outdoor tests in small screen cages, each cage with 20 beetles (10 males, 10 females) and two plants (saltcedar, *T. ramosissima* × *T. chinensis*, and athel, *T. aphylla*), $n = 5$. For each day postrelease, individual means followed by the same letter within and between the Crete, Tunisia, and Uzbekistan columns are not significantly different (Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

parisons of population growth statistics were made by randomly dividing females into four groups (replicates) of three to four females for each of the four treatments.

Statistical Analyses

Data on the number or percentage of adults or eggs per location for the adult no-choice and choice tests were analyzed using a protected Kruskal-Wallis test performed on the ranks of the data (PROC GLM; SAS Institute 1999), including a repeated-measures analysis for the no-choice and paired-choice test (Srivastava and Carter 1983). All other data were subjected to analysis of variance (ANOVA; PROC MIXED, SAS Institute 1999). Data on percentage survival were arcsine squareroot transformed. For all tests, means were separated using Fisher's protected least significant difference (LSD) test (LSMEANS; SAS Institute 1999) or preplanned contrasts (CONTRAST; SAS Institute 1999).

Results

Adult Tests

No-Choice Test. The distribution of adult *D. elongata* among locations within cages differed among Crete, Tunisia, and Uzbekistan populations over the 3 d of the test (Day × Location × Beetle population: $F = 2.18$; df = 8,42; $P = 0.049$; Table 1). On the first day postrelease, fewer Uzbekistan beetles were found on the plants, for both athel and saltcedar (*T. ramosissima x T. chinensis*), than Tunisia beetles. Also, more Uzbekistan adults were located on the cage walls compared with beetles from Crete and Tunisia (Table 1). However, the majority of adults, including several mating pairs, were present on the *Tamarix* test plants, which was greater than the number of adults on the cage walls (contrast: $F = 6.10$; df = 2,24; $P < 0.008$). By the third day, similarly high numbers of adult beetles were present on both athel and saltcedar, regardless of beetle population (Table 1). In our caged no-choice test, a similar number of eggs were laid on athel and saltcedar by all three *D. elongata* populations. More eggs were laid on the plants (287 ± 88 eggs) than on the cage walls (67 ± 56 eggs, $F = 121.51$; df = 1,24; $P < 0.001$).

Paired-Choice Test. The distribution of adult *D. elongata*, when given a choice between saltcedar and athel, differed among the three beetle populations over the duration of the test (Day × Treatment: $F = 2.91$; df = 22,94; $P < 0.001$; Table 2). On the first day postrelease, more adult beetles from Crete and Uzbekistan were present on the saltcedar plants than on the athel plants or cage walls. In contrast, similar numbers of Tunisia adults were present on athel and saltcedar (Table 2). Mating pairs were observed on both saltcedar and athel. By the third day postrelease, more *D. elongata* adults were present on saltcedar compared with athel for all beetle populations (Table 2). In addition, similar numbers of Uzbekistan beetles were located on the saltcedar plant and cage walls, which was not the case for the other two populations (Table 2). Oviposition differed among the populations of *D. elongata* (Fig. 2). Crete and Tunisia beetles laid more eggs on saltcedar, averaging 192 or 228 eggs/plant, than on athel or the cage walls. Athel received less than one half the number of eggs compared with saltcedar. More eggs also were laid on athel (70 or 92 eggs/plant) than on the cage walls (16 or 35 eggs; Fig. 2). In contrast, for Uzbekistan beetles, we found no differences in the number of eggs among the two *Tamarix* plants and the cage walls (Fig. 2). The number of eggs laid on the cage walls by Uzbekistan beetles was much higher, 151 eggs on average, than that laid by Crete and Tunisia beetles (Fig. 2).

Cage Size. This test compared adult colonization and oviposition by Crete *D. elongata* between outdoor

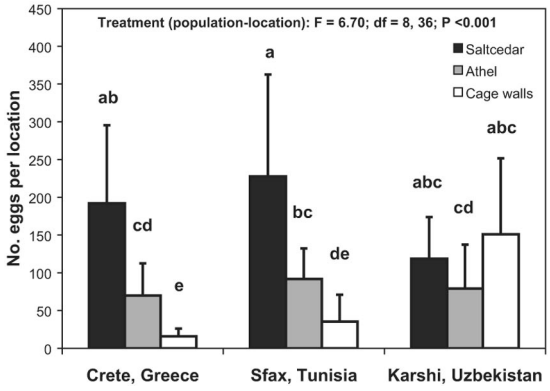

Fig. 2. Oviposition by *D. elongata* (mean + SD) from three different populations after 3 d on saltcedar and athel: paired-choice tests, Temple, TX, June 2004. Bars denoted by the same letter are not significantly different (one-way Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected LSD, $P > 0.05$).

cages of two different sizes, 0.3 and 18 m^3, for the no-choice (Table 3) and paired-choice tests (Table 4; Fig. 3). In the no-choice test, the identity of the test plant (athel or saltcedar) did not influence the location of adults among the plants or cage walls. The size of the cage did have an effect on how adult beetles were distributed over the 3 d of the test (Day × Location × Cage size: $F = 8.31$; df = 4,13; $P < 0.002$; Table 3). Fewer Crete adults were present on test plants in the large cages, and more were unaccounted, compared with the small cages 1 d after release. More adults were present on the plants than on the cage walls (contrast: $F = 9.86$; df = 1,16; $P < 0.007$). By 3 d after release, a similar number of adults were present on the test plants for the two cage sizes, although more were still unaccounted in the large cages than in the small cages (Table 3). Although more total eggs were laid in the small cages (324.6 ± 57.0) than in the large cages (195.8 ± 92.4; $F = 20.16$; df = 1,16; $P < 0.001$), an increase in cage size did not change the pattern of egg-laying by Crete beetles in the no-choice test. A similar number of eggs were laid on athel and saltcedar, and more eggs were laid on the test plants (239.8 ± 92.5) than on the cage walls (20.4 ± 28.1; $F = 90.16$; df = 1,16; $P < 0.001$) for both small and large cages.

For the paired-choice test, the location of adults varied by cage size and day postrelease (Day × Treatment: $F = 4.99$; df = 14,62; $P < 0.001$). On the first day after release, more Crete adults were present on the saltcedar plant than elsewhere in the small cages, whereas very few adults were found on either plant in the large cages (Table 4). By 3 d after release, the pattern of adult colonization was similar between the two cage sizes, with more adults present on the salt-

Table 3. Distribution of adult *D. elongata* (mean ± SD) from Crete in different cage sizes 1 and 3 d after release in no-choice tests with saltcedar or athel: Temple, TX, May–June 2004

Location of adults	No. adults	
	Small cage	Large cage
1 d after release		
Plant	15.1 ± 2.3a	8.8 ± 4.0b
Cage walls	1.2 ± 1.2a	2.9 ± 2.8a
Dead/unaccounted	3.7 ± 2.7b	8.3 ± 2.8a
3 d after release		
Plant	17.1 ± 2.5a	14.4 ± 3.2a
Cage walls	2.7 ± 2.4a	0.9 ± 1.3a
Dead/unaccounted	0.2 ± 0.4b	4.7 ± 2.2a

Outdoor tests in either small screen cages (68 by 53 by 85 cm) or large field cages (3 by 3 by 2 m), each cage with 20 beetles (10 males, 10 females) and one test plant (saltcedar, *T. ramosissima* × *T. chinensis*, or athel, *T. aphylla*), $n = 5$. Means are averaged over saltcedar and athel, which was not a significant factor (test plant, $P > 0.05$). For each day postrelease and location, means within a row followed by the same letter are not significantly different (Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

Table 4. Distribution of adult *D. elongata* (mean ± SD) from Crete in different cage sizes 1 and 3 d after release in paired-choice tests with saltcedar and athel: Temple, TX, June 2004

Location of adults	No. adults	
	Small cages	Large cages
1 d after release		
Saltcedar	10.2 ± 4.0ab	1.8 ± 2.5de
Athel	2.8 ± 1.8cd	0.4 ± 0.5e
Cage walls	2.6 ± 3.1cde	5.4 ± 3.9bc
Dead/unaccounted	4.4 ± 2.5cd	12.4 ± 4.9a
3 d after release		
Saltcedar	14.0 ± 1.6a	10.2 ± 4.8a
Athel	4.0 ± 1.4b	4.6 ± 4.0b
Cage walls	1.8 ± 1.1c	1.0 ± 1.4c
Dead/unaccounted	0.2 ± 0.4c	4.2 ± 1.9b

Outdoor tests in either small screen cages (68 by 53 by 85 cm) or large field cages (3 by 3 by 2 m), each cage with 20 beetles (10 males, 10 females) and two plants (saltcedar, *T. ramosissima* × *T. chinensis*, and athel, *T. aphylla*), $n = 5$. For each day postrelease, individual means followed by the same letter within and between the small and large cage columns are not significantly different (Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

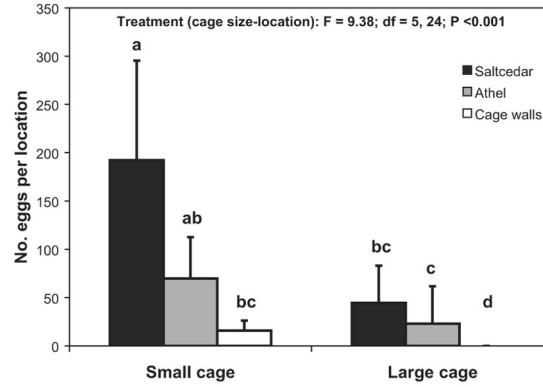

Fig. 3. Oviposition by Crete *D. elongata* (mean + SD) in two sizes of cage after 3 d on saltcedar and athel: paired-choice tests, Temple, TX, June 2004. Bars denoted by the same letter are not significantly different (one-way Kruskal-Wallis test on ranks with mean rank values separated by Fisher's protected LSD, $P > 0.05$).

cedar plants than on athel or the cage walls (Table 4). As in the previous no-choice test, more total eggs were laid in the small cages than in the large cages over 3 d (small versus large cage contrast: $F = 21.64$; df = 1,24; $P < 0.001$), but the pattern of oviposition by Crete *D. elongata* was similar between the two cage sizes: a statistically similar number of eggs were laid on saltcedar and athel, and more eggs generally were laid on the two test plants than on the cage walls (Fig. 3).

Multiple-Choice Test. This test compared adult preference by Crete, Tunisia, and Uzbekistan *D. elongata* for different accessions of athel and various species or hybrids of saltcedar in large outdoor cages (Table 5). The percentage distribution of adults within each of the various locations (six different test plants, cage walls, and ground) was similar among the three beetle types, except for a lower percentage of Crete beetles on *T. ramosissima* × *T. canariensis*/*T. gallica* than Tunisia and Uzbekistan beetles. The percentage of adults present on the four saltcedar entries was greater than on the two athel entries for all three beetle types, with the exception of Tunisia beetles colonizing *T. canariensis*/*T. gallica.* Adult colonization of the athel accessions also was not different than that of the cage walls. Oviposition was greater on saltcedar than on athel averaged over the three beetle populations (contrast: $F = 76.54$; df = 1,104; $P < 0.001$). As in the paired-choice test, athel plants received on average only one fourth to one half the amount of eggs as saltcedar plants. However, the response of the three beetle types was more variable when considering individual accessions of saltcedar and athel. For *D. elongata* from Tunisia and Uzbekistan, greater percentages of eggs were found on the different saltcedar entries, with the exception of *T. canariensis*/*T. gallica*, compared with athel. In contrast, Crete *D. elongata* showed no differences in oviposition among three of the four saltcedars and the athel from Phoenix, AZ. In all cases, oviposition on the *T. ramosissima* × *T. chinensis* hybrid, the most widespread *Tamarix* type, was significantly greater than on either of the two athel accessions. The saltcedar *T. canariensis*/*T. gallica* usually received a smaller percentage of eggs than the other saltcedars used in this test for all beetle populations. In all cases, the two athel accessions received similar percentages of eggs within a beetle population. However, Crete beetles did lay a higher percentage of eggs on athel (Phoenix, AZ) compared with Tunisia and Uzbekistan beetles.

Table 5. Adult colonization and oviposition by *D. elongata* from three populations on saltcedar and athel: multiple-choice tests in large outdoor cages, Temple, TX, July–Aug. 2004

Test plant, cage walls, ground[a]	Percent adults observed or eggs laid (mean ± SD) per location (total per test)					
	Adults			Eggs		
	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan	Crete, Greece	Sfax, Tunisia	Karshi, Uzbekistan
T. ramosissima × *T. chinensis* Big Spring, TX (AY090385, AY090386)	20.6 ± 2.4abcd (136)	21.1 ± 3.2abcd (87)	23.5 ± 5.4abc (70)	21.6 ± 5.8ab (3604)	22.8 ± 10.7abc (2860)	28.0 ± 7.2a (3110)
T. parviflora Las Cruces, NM	19.1 ± 3.2bcde (139)	21.0 ± 5.7abcd (85)	15.5 ± 9.0de (47)	19.5 ± 14.3abc (3257)	26.6 ± 6.0a (2897)	18.9 ± 7.8bcd (2187)
T. ramosissima × *T. canariensis*/*T. gallica* Seymour, TX (AY090385, AY090437)	18.0 ± 8.8cde (126)	23.8 ± 4.9ab (103)	27.5 ± 6.8a (82)	19.2 ± 4.9abc (3418)	19.2 ± 9.5bcd (2240)	25.5 ± 5.1a (2718)
T. canariensis/*T. gallica* Texas City, TX (AY090398, AY090437)	19.3 ± 5.9bcde (136)	14.6 ± 3.6ef (61)	20.3 ± 5.9abcde (64)	13.9 ± 5.3def (2556)	11.5 ± 2.3efg (1550)	12.7 ± 7.6def (1463)
T. aphylla (athel) Phoenix, AZ	9.3 ± 5.2fg (56)	6.5 ± 2.3gh (25)	4.0 ± 4.1gh (13)	15.1 ± 6.8cde (2180)	8.1 ± 5.7fg (901)	5.1 ± 4.2gh (507)
T. aphylla (athel) Encino, TX	8.1 ± 7.0fgh (52)	8.7 ± 6.9fg (38)	5.4 ± 3.9gh (20)	10.8 ± 4.8efg (1509)	11.8 ± 6.2ef (1409)	8.5 ± 2.7fg (813)
Cage walls	5.6 ± 4.6gh (35)	3.7 ± 2.5gh (19)	3.7 ± 3.7hi (13)	0.0 ± 0.0i (0)	0.1 ± 0.2i (17)	1.2 ± 1.3hi (135)
Ground/weeds	0.0 ± 0.0j (0)	0.5 ± 0.7ij (2)	0.2 ± 0.4j (1)	0.0 ± 0.0i (0)	0.0 ± 0.0i (0)	0.2 ± 0.4i (21)

Outdoor tests in large cages, $n = 5$ or 6 (Crete only) with each replicate (representing a 3- to 6-d period) the average of four quadrats in a cage. For each life stage, individual means followed by the same letter within and between the Crete, Tunisia, and Uzbekistan columns are not significantly different (one-way Kruskal-Wallis Test on ranks with mean rank values separated by Fisher's protected least significant difference test, $P > 0.05$).

[a] Molecular identification by J. F. Gaskin (USDA-ARS, Sidney, MT). × denotes a hybrid. Accession numbers, if available, represent either the two haplotypes of heterozygous pepC genes or the two identical haplotypes of homozygous pepC genes (National Institute of Health's GenBank).

Larval Development and Adult Fecundity

Testing of the suitability of athel and saltcedar for *D. elongata* from Crete revealed no differences between the two diets in larval/pupal survival or the duration of larval and pupal stages, except for a minor difference in the second stadium (Table 6). Larvae fed athel produced slightly smaller male and female adults than larvae fed saltcedar (Table 6). The type of larval diet did not influence any measure of female reproduction, days for egg hatch, or adult longevity (Table 7). However, an adult diet of athel resulted in a longer

Table 6. Immature development and survival and adult size of *D. elongata* from Crete fed saltcedar or athel, Temple, TX, 2004

Stage	Duration (d)	
	Saltcedar	Athel
First instar	3.8 ± 0.1a (99)	3.8 ± 0.1a (100)
Second instar	3.1 ± 0.1a (97)	2.9 ± 0.1b (97)
Third instar		
Active	3.9 ± 0.1a (92)	3.9 ± 0.1a (94)
Prepupa	2.5 ± 0.1a (84)	2.5 ± 0.1a (87)
Pupa	4.6 ± 0.1a (80)	4.7 ± 0.1a (81)
Total (neonate to adult)	17.8 ± 0.1a (80)	17.7 ± 0.1a (81)
	Percent survival (neonate to adult)	
	85.0 (72.5–93.7)a (10)	84.2 (72.5–93.1)a (10)
Sex	Left elytral length (mm)	
Female	5.19 ± 0.04 (47)	5.09 ± 0.05 (33)
Male	4.57 ± 0.05 (29)	4.46 ± 0.04 (42)
Factors for length	*F*-value; df; *P* value	
Diet	5.12; 1,147; 0.025	
Sex	193.35; 1,147; <0.001	
Diet × sex	0.00; 1,147; 0.962	

Larval-pupal development based on an initial 100 randomly selected larvae, fed saltcedar (*T. ramosissima* × *T. chinensis*) or athel (*T. aphylla*) at 28°C and checked daily. Values are mean ± SE (*n*) except percent survival, which are back-transformed means from arcsine square root values, followed by 95% CIs in parentheses. Within each row (except length), means followed by the same letter are not significantly different (Fisher's protected least significant difference test, $P > 0.05$).

preoviposition period, longer total longevity for both males and females, and a smaller number of eggs per egg mass than adults fed saltcedar (Table 7). The duration of the oviposition period, total eggs or egg masses per female and days to egg hatch were not significantly affected by the adult diet (Table 7). Crete beetles fed a larval–adult diet of athel–saltcedar had a slightly lower hatch rate of eggs compared with beetles that had received a saltcedar–saltcedar diet; otherwise, percentage hatch was similar (Table 7). Although the larval diet had no effect on any population parameter calculated for Crete *D. elongata*, the adult diet did have a significant effect. Adults fed athel had a longer mean generation time (T), at least 6 d longer, a 13% lower innate capacity for increase (r_m), and a slightly longer population doubling time (DT) than when adults were fed saltcedar (Table 8).

Discussion

Adult Acceptance

The acceptability of athel as a host plant to adults of the three populations of the leaf beetle *D. elongata* that we tested (from Tunisia, Crete, and Uzbekistan) was similar to saltcedar in a no-choice setting and variable in a choice setting. In previous multiple-choice tests with these same populations and additional ones from China and Kazakhstan, athel usually received one third to one half the amount of eggs than most saltcedar accessions (DeLoach et al. 2003, Lewis et al. 2003a, Milbrath and DeLoach 2006). Notable exceptions in which no preference between saltcedar and athel occurred involved the Uzbekistan beetle in large cage tests and *D. elongata* from Crete and Fukang, China, in small cage tests (Milbrath and DeLoach 2006). In this study, Uzbekistan beetles did show a higher degree of preference for most saltcedars over athel in the large cage, multiple-choice test, although they did not discriminate between saltcedar and athel

Table 7. Adult development and fecundity of *D. elongata* from Crete fed saltcedar or athel, Temple, TX, 2004

Larval diet	Adult diet	Duration (d)				
		Preoviposition period	Oviposition period	Total longevity, females	Total longevity, males	Egg hatch
Athel	Athel	8.1 ± 0.5 (11)	26.6 ± 3.8 (11)	31.9 ± 5.7 (13)	47.0 ± 5.5 (14)	5.2 ± 0.1 (25)
Athel	Saltcedar	5.9 ± 0.4 (15)	18.4 ± 3.3 (15)	26.1 ± 5.3 (15)	31.2 ± 5.0 (17)	5.2 ± 0.1 (25)
Saltcedar	Athel	8.5 ± 0.5 (12)	20.6 ± 3.7 (12)	26.6 ± 5.5 (14)	57.5 ± 5.7 (13)	5.2 ± 0.1 (25)
Saltcedar	Saltcedar	6.9 ± 0.4 (14)	15.0 ± 3.4 (14)	22.1 ± 5.3 (15)	34.9 ± 5.3 (15)	5.3 ± 0.1 (25)
Factor[a]		*F*-value; df; *P* value				
Larval diet		2.55; 1,48; 0.117	1.78; 1,48; 0.188	0.11; 1,108; 0.746		0.57; 1,96; 0.452
Adult diet		16.96; 1,48; <0.001	3.81; 1,48; 0.057	10.27; 1,108; <0.002		0.57; 1,96; 0.452
Sex		—	—	17.54; 1,108; <0.001		—

Larval diet	Adult diet	Total eggs/female	Total egg masses/female	Eggs/mass	Percent hatch
Athel	Athel	328.0 ± 60.4 (13)	24.3 ± 4.5 (13)	13.8 ± 0.6 (11)	84.9 (79.3–89.8) (11)
Athel	Saltcedar	305.1 ± 56.2 (15)	20.5 ± 4.2 (15)	15.5 ± 0.5 (15)	79.6 (74.3–84.4) (15)
Saltcedar	Athel	278.1 ± 58.2 (14)	19.9 ± 4.4 (14)	14.5 ± 0.6 (12)	79.8 (73.9–85.1) (12)
Saltcedar	Saltcedar	219.7 ± 56.2 (15)	14.3 ± 4.2 (15)	16.0 ± 0.5 (14)	86.5 (81.7–90.6) (14)
Factor		*F*-value; df; *P* value			
Larval diet		1.37; 1,53; 0.247	1.49; 1,53; 0.228	1.17; 1,48; 0.285	0.14; 1,48; 0.714
Adult diet		0.50; 1,53; 0.484	1.20; 1,53; 0.278	9.36; 1,48; 0.004	0.09; 1,48; 0.766
Diet × diet		0.09; 1,53; 0.760	0.04; 1,53; 0.835	0.04; 1,48; 0.852	5.56; 1,48; 0.023

Adult reproduction and longevity based on an initial 15 mating pairs fed a combination of athel (*T. aphylla*) and/or saltcedar (*T. ramosissima* × *T. chinensis*) as larvae and adults, and egg development based on one to four egg masses randomly sampled per female, reared at 28°C, and checked daily. Values are mean ± SE (*n*) except percent hatch, which are back-transformed means from arcsine square root values, followed by 95% CIs in parentheses.

[a] No interaction terms were significant ($P > 0.05$).

Table 8. Population growth statistics (mean ± SE) for *D. elongata* from Crete fed saltcedar or athel, Temple, TX, 2004

Larval diet	Adult diet	Net reproductive rate (R_o)	Mean generation time (d, T)	Innate capacity for increase (r_m)	Population doubling time (d, DT)
Athel	Athel	105.5 ± 14.6	43.5 ± 1.4	0.107 ± 0.004	6.6 ± 0.2
Athel	Saltcedar	101.4 ± 14.6	36.5 ± 1.4	0.126 ± 0.004	5.6 ± 0.2
Saltcedar	Athel	94.7 ± 14.6	42.0 ± 1.4	0.109 ± 0.004	6.4 ± 0.2
Saltcedar	Saltcedar	79.9 ± 14.6	35.6 ± 1.4	0.121 ± 0.004	5.8 ± 0.2
Factor		*F*-value; df; *P* value			
Larval diet		1.22; 1,12; 0.291	0.75; 1,12; 0.403	0.05; 1,12; 0.835	0.01; 1,12; 0.916
Adult diet		0.42; 1,12; 0.531	22.13; 1,12; <0.001	14.41; 1 12; 0.003	12.71; 1,12; 0.004
Diet × diet		0.13; 1,12; 0.721	0.05; 1,12; 0.822	0.78; 1,12; 0.396	0.57; 1,12; 0.464

Adults reared on a combination of athel (*T. aphylla*) and/or saltcedar (*T. ramosissima* × *T. chinensis*) as larvae and adults at 28°C. Calculations based on values of 22.9 (athel–athel, athel–saltcedar), 23.0 (saltcedar–athel), and 23.1 d (saltcedar–saltcedar) preadult development, $n = 4$ groups of females with three to four females per group.

in the small cage, paired-choice test. In contrast, the Crete beetle showed a preference for saltcedar in the paired-choice test but not in the multiple-choice test. The Tunisia beetle was the only population tested that consistently displayed an oviposition preference for saltcedar in choice tests. Nevertheless, when athel was offered by itself, all populations of *D. elongata* colonized and laid eggs on athel at a level comparable with that of saltcedar. Adult *D. elongata*, which are considered to be the most discriminating stage when it comes to host plant selection, especially when ovipositing (DeLoach et al. 2003, Lewis et al. 2003a), apparently find athel as acceptable as saltcedar under confined, no-choice conditions. However, no-choice cage tests are not realistic in the sense that the insects cannot leave the experimental arena—a current constraint of quarantine tests that may produce false positive results but one that minimizes false-negative results (Cullen 1990, Marohasy 1998, Heard 2000). Open field tests are one option to better understand the acceptability of nontarget plants (discussed below).

We compared host plant selection in small versus large cages, using the same number of adult beetles, to determine if the selection of athel relative to saltcedar decreased in larger cages, which could indicate a step toward even less selection of athel in the open field. We consider large cages, primarily used for multiple-choice tests, as providing a more realistic setting as the beetles are less confined (Cullen 1990, Heard 2000). However, cage size did not affect the pattern of oviposition between athel and saltcedar in either the no-choice or paired-choice tests. The reduced oviposition we observed in the large cages, which were 60 times larger in volume than the small cages, seemed to be caused by a substantial delay in colonization of the test plants by the adult beetles during the 3-d period we imposed. Therefore, tests in large cages using small numbers of adults need to be conducted for a longer period compared with small cages. The use of smaller cages is often preferred, assuming it is appropriate given the biology of the insect (Briese et al. 2002), given constraints on space and the desire to test as many plants as possible simultaneously. For purposes of laboratory or greenhouse quarantine testing, small cages did not seem to bias our results in this and previous tests (Milbrath and DeLoach 2006).

Larval/Adult Suitability

The suitability of athel, and indeed all *Tamarix* species and hybrids tested, for larval development in *D. elongata* was known previously, although usually only percentage survival was recorded (DeLoach et al. 2003, Lewis et al. 2003a, 2003b, Milbrath and DeLoach 2006). Our more detailed examination showed that the only apparent negative effect of a larval diet of athel was a slight reduction in adult size for Crete beetles compared with larvae fed saltcedar. Adults that were fed athel, regardless of the previous larval diet, lived longer and showed a trend toward a longer oviposition period than their saltcedar-fed counterparts, resulting in similar lifetime fecundities. However, an adult diet of athel also negatively affected some aspects of reproduction, in particular delaying the start of oviposition and reducing the size of egg masses. As a result, population growth rate decreased by 13% and the population doubling time increased slightly. We do not know if these results would change if the beetles were reared on intact athel plants in the field. Nevertheless, our laboratory results contrast with the greater negative effect that a lifetime diet of athel had on *D. elongata deserticola* from Fukang, China (Lewis et al. 2003b), although they concluded that *D. elongata deserticola* still would be able to sustain a population on athel.

Risk to Athel

Kovalev (1995) stated that athel generally has a different insect fauna than saltcedar and that specialist insects of saltcedar species of *Tamarix* would be unlikely to develop on athel. However, he restricted his comments to those insects that are geographically isolated from the range of athel. Athel overlaps with the distribution of *D. elongata*, from North Africa to Pakistan (Lopatin 1977, Baum 1978). We have a field collection record of *D. elongata* reared from athel in Pakistan. Based on known morphological differences among the studied populations, the beetle was similar to the Uzbekistan population (J. L. Tracy, personal communication). Therefore, some field use of athel by *D. elongata* does occur in the Old World, although to what extent is unclear.

The acceptability of athel for oviposition and the ability of *D. elongata* to develop, survive, and reproduce on athel in quarantine tests indicates that athel is at least at a moderate risk to damage in North America, although we cannot predict what level of damage may occur in the open field. Because of the current value placed on athel as an ornamental shade tree and windbreak, especially in northern Mexico, we would like the approval of Mexican scientists, natural areas managers, and authorities before releases of *D. elongata* are made along the Rio Grande, TX. However, saltcedar has invaded many areas in northern Mexico, and biological control is desired there as well as in the United States, creating a similar conflict of interest for Mexico. We are attempting to resolve this issue by conducting uncaged, open-field tests involving the transplanting of athel into natural stands of saltcedar in which Crete beetles have been released. Two field experiments have been initiated—one in 2004 involving an isolated patch of saltcedar near Kingsville, TX, and the second in 2005 with a more extensive stand of saltcedar at Big Spring, TX. Currently, the beetles are only established outside of field cages at Big Spring, and it may be a few years before definitive answers are available. The use of a different biological control agent may be needed to minimize damage to athel, assuming such an agent will still attack the complex of saltcedar species and hybrids present.

Athel is, however, an exotic ornamental, and not all workers consider protecting such plants a necessary consideration for weed biological control programs, given the possibility of finding suitable replacements (Pemberton 2002). Indeed, given that the related saltcedars are highly invasive, it is probable that athel could eventually show invasive tendencies in North America, as it has in Australia (Griffen et al. 1989). Initially, only a few locations of naturalized stands of athel were known, the largest at the Salton Sea and along the lower Colorado River, CA (DeLoach et al. 2003). More recently, additional sites have been identified near Coalinga, CA (Dorn 1986); Niland, CA; Buckeye, AZ; and Rio Grand Village and Mercedes, TX (C.J.D., unpublished data). These are believed to have propagated vegetatively from branches, because athel seed is dropped in late summer when limited moisture greatly limits germination except during summer floods (Danin 1981, Dorn 1986, Griffen et al. 1989). However, reproduction by seed has been confirmed at Lake Mead, NV (Barnes 2003), and hybridization is also occurring between athel and the invasive saltcedars *T. ramosissima* and *T. chinensis* (Gaskin and Shafroth 2005). The goal of minimizing risk to athel could conceivably change to that of controlling athel should it become an invasive plant in North America in the future.

Acknowledgments

We thank our colleagues who collected the beetles—R. Sobhian, A. Kirk (USDA–ARS European Biological Control Laboratory, Montferrier-sur-Lez, France), J. Kashefi (USDA–ARS EBCL, Thessaloniki, Greece), M. Cristofaro (ENEA C.R. Casaccia, Rome, Italy), and R. Carruthers (USDA–ARS, Albany, CA); J. Tracy, T. Robbins, and N. Messer (USDA–ARS, Temple, TX) for technical assistance; P. Moran (USDA–ARS, Weslaco, TX) for supplying athel foliage; D. Bean (University of California-Davis) and T. Wang (USDA–ARS, Albany, CA) for supplying beetles; J. Gaskin (USDA–ARS, Sidney, MT) for DNA analysis of *Tamarix* species; J. Sanabria (Texas Agricultural Experiment Station, Temple, TX) for statistical advice; and anonymous reviewers for comments. This research was funded in part by USDA-CSREES-IFAFS Grant 00-52103–9647.

References Cited

Arnett, A. E., and S. M. Louda. 2002. Re-test of *Rhinocyllus conicus* host specificity, and the prediction of ecological risk in biological control. Biol. Conserv. 106: 251–257.

Barnes, P. L. 2003. Reproductive and population characteristics of *Tamarix aphylla* at Lake Mead National Recreation Area, Nevada. MS thesis, University of Nevada Las Vegas, NV.

Baum, B. R. 1967. Introduced and naturalized tamarisks in the United States and Canada (Tamaricaceae). Baileya 15: 19–25.

Baum, B. R. 1978. The genus *Tamarix.* Israel Academy of Sciences and Humanities, Jerusalem, Israel.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26.

Briese, D. T., A. Walker, W. J. Pettit, and J.-L. Sagliocco. 2002. Host-specificity of candidate agents for the biological control of *Onopordum* spp. thistles in Australia: an assessment of testing procedures. Biocontrol. Sci. Technol. 12: 149–163.

Crins, W. L. 1989. The Tamaricaceae in the southeastern United States. J. Arnold Arbor. Harv. Univ. 70: 403–425.

Cullen, J. M. 1990. Current problems in host-specificity screening, pp. 27–36. *In* E. S. Delfosse (ed.), Proceedings of the VII international symposium on biological control of weeds. Instituto Sperimentale per la Patologia Vegetale, Rome, Italy.

Danin, A. 1981. Ecological factors affecting the distribution of *Tamarix aphylla* (L.) Karst. in Israel and Sinai. La-Yaaran 31: 8–11.

DeLoach, C. J. 2004. *Tamarix aphylla.* Crop protection compendium database [CD-ROM]. CAB International, Wallingford, UK.

DeLoach, C. J., R. I. Carruthers, J. E. Lovich, T. L. Dudley, and S. D. Smith. 2000. Ecological interactions in the biological control of saltcedar (*Tamarix* spp.) in the United States: toward a new understanding, pp. 819–873. *In* N. R. Spencer (ed.), Proceedings of the X international symposium on biological control of weeds. Montana State University, Bozeman, MT.

DeLoach, C. J., P. A. Lewis, J. C. Herr, R. I. Carruthers, J. L. Tracy, and J. Johnson. 2003. Host specificity of the leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (*Tamarix:* Tamaricaceae) in the Western United States. Biol. Control 27: 117–147.

DeLoach, C. J., R. I. Carruthers, T. L. Dudley, D. Eberts, D. J. Kazmer, A. E. Knutson, D. W. Bean, J. Knight, P. A. Lewis, L. R. Milbrath, J. L. Tracy, N. Tomic-Carruthers, J. C. Herr, G. Abbott, S. Prestwich, G. Harruff, J. H. Everitt, D. C. Thompson, I. Mityaev, R. Jashenko, B. Li, R. Sobhian, A. Kirk, T. O. Robbins, and E. S. Delfosse. 2004. First results for control of saltcedar (*Tamarix* spp.) in the open field in the Western United States, pp. 505–513. *In*

J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, and J. K. Scott (eds.), Proceedings of the XI international symposium on biological control of weeds. CSIRO Entomology, Canberra, Australia.

DiTomaso, J. M. 1998. Impact, biology, and ecology of saltcedar (*Tamarix* spp.) in the Southwestern United States. Weed Technol. 12: 326–336.

Dorn, M. D. 1986. The biogeography and ecology of *Tamarix* in California. MA thesis, University of California, Los Angeles, CA.

Gaskin, J. F., and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proc. Natl. Acad. Sci. U.S.A. 99: 11,256–11,259.

Gaskin, J. F., and B. A. Schaal. 2003. Molecular phylogenetic investigation of U.S. invasive *Tamarix*. Syst. Bot. 28: 86–95.

Gaskin, J. F., and P. B. Shafroth. 2005. Hybridization of *Tamarix ramosissima* and *T. chinensis* (saltcedars) with *T. aphylla* (athel) (family Tamaricaceae) in the Southwestern USA determined from DNA sequence data. Madrono 52: 1–10.

González, L. L. de Léon, and R. Vásquez Aldape. 1991. Distribución, abundancia y utilización del pinabete (*Tamarix* spp.) en el Norte de México. Universidad Autónoma Agraria Antonio Narro, Departamento de Recursos Naturales Renovables, Buenavista, Saltillo, Coahuila, México.

Griffin, G. F., D. M. Stafford Smith, S. R. Morton, G. E. Allan, and K. A. Masters. 1989. Status and implications of the invasion of tamarisk (*Tamarix aphylla*) on the Finke River, Northern Territory, Australia. J. Environ. Manag. 29: 297–315.

Heard, T. A. 2000. Concepts in insect host-plant selection behavior and their application to host specificity testing, pp. 1–10. *In* R. Van Driesche, T. Heard, A. McClay, and R. Reardon (eds.), Proceedings of session: host-specificity testing of exotic arthropod biological control agents—the biological basis for improvement in safety. X international symposium on biological control of weeds. U.S. Department of Agriculture Forest Service, Morgantown, WV.

Horton, J. S. 1964. Notes on the introduction of deciduous tamarisk. U.S. Department of Agriculture Forest Service, Ft. Collins, CO.

Kovalev, O. V. 1995. Co-evolution of the tamarisks (Tamaricaceae) and pest arthropods (Insecta; Arachnida: Acarina), with special reference to biological control prospects. Pensoft Publishers, Moscow, Russia.

Lewis, P. A., C. J. DeLoach, J. C. Herr, T. L. Dudley, and R. I. Carruthers. 2003a. Assessment of risk to native *Frankenia* shrubs from an Asian leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae), introduced for biological control of saltcedars (*Tamarix* spp.) in the western United States. Biol. Control 27: 148–166.

Lewis, P. A., C. J. DeLoach, A. E. Knutson, J. L. Tracy, and T. O. Robbins. 2003b. Biology of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae), an Asian leaf beetle for biological control of saltcedars (*Tamarix* spp.) in the United States. Biol. Control 27: 101–116.

Lopatin, I. K. 1977. The leaf-beetles of Central Asia and Kazakhstan. Nauka Publishing, Leningrad, Russia.

Marohasy, J. 1998. The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. Biocontrol News Inf. 19: 13N–20N.

Milbrath, L. R., and C. J. DeLoach. 2006. Host specificity of different populations of the leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.). Biol. Control 36: 32–48.

Pemberton, R. W. 2002. Selection of appropriate future target weeds for biological control, pp. 375–386. *In* R. Van Driesche, S. Lyon, B. Blossey, M. Hoddle, and R. Reardon (eds.), Biological control of invasive plants in the eastern United States. U.S. Department of Agriculture Forest Service, Morgantown, WV.

Robinson, T. W. 1965. Introduction, spread and areal extent of saltcedar (*Tamarix*) in the western states. U.S. Geological Survey, Washington, DC.

SAS Institute. 1999. SAS/STAT user's guide, version 8th ed. SAS Institute, Cary, NC.

Srivastava, M. S., and E. M. Carter. 1983. An introduction to applied multivariate statistics. North Holland, New York.

U.S. Department of Agriculture–Natural Resources Conservation Service. 2005. The PLANTS database, version 3.5 (http://plants.usda.gov).

Received for publication 28 October 2005; accepted 2 July 2006.